

AMENDMENTS TO THE SPECIFICATION

On page 1, immediately following the title, please insert the following continuing data:

---This is a § 371 of PCT/GB99/02720, filed August 17, 1999, and published March 2, 2000, as WO 00/11196, which claims priority of GB Application No. 9818003.7, filed August 18, 1998, both of which are incorporated herein by reference in their entirety.---

Please replace the Brief Description of Figures 1 through 15 beginning on page 7, line 25, through page 8, line 15, with the following rewritten Brief Description of the figures:

--- Figure 1 shows the polynucleotide and corresponding amino acid sequences for A) Dm1 (~~SEQ ID No 5~~ polynucleotide: SEQ ID NO: 5), (amino acid: SEQ ID NO: 12) and B) Dm2.18 (~~SEQ ID No 1~~ polynucleotide: SEQ ID NO: 1), (amino acid: SEQ ID NO: 8).

Figure 2 shows the polynucleotide and corresponding amino acid sequences for A) Dm2.1 (~~SEQ ID No 2~~ polynucleotide: SEQ ID NO: 2), (amino acid: SEQ ID NO: 9) and B) Dm2.3 (~~SEQ ID No 3~~ polynucleotide: SEQ ID NO: 3), (amino acid: SEQ ID NO: 10).

Figure 3 shows the polynucleotide (SEQ ID NO: 4) and corresponding amino acid (SEQ ID NO: 11) sequence for Dm2.5, (~~SEQ ID No 4~~),

Figure 4 shows a diagrammatic map of plasmids pMJB1, pDmAMPD and pDmAMPE₂[:]

Figure 5 shows a diagrammatic map of plasmid pFAJ3106₂[:]

Figure 6 shows a diagrammatic map of plasmid pFAJ3109₂

Figure 7 shows the nucleotide sequence (SEQ ID NO: 34) and the corresponding amino acid sequence (SEQ ID NO: 35) between the XhoI and SacI sites of pFAJ3106₂[:]

Figure 8 shows the nucleotide sequence (SEQ ID NO: 36) and the corresponding amino acid sequence (SEQ ID NO: 37) between the XhoI and SacI sites of pFAJ3109.[;]

Figure 9 shows a diagrammatic map of plasmid pZPS38.[;]

Figure 10 shows a diagrammatic map of plasmid pZPS34.[;]

Figure 11 shows a diagrammatic map of plasmid pZPS35.[;]

Figure 12 shows a diagrammatic map of plasmid pZPS37.

Figure 13 shows a plan of the construction of the Dm-AMP gene.

Figure 14 shows one predicted polynucleotide sequence for DmAMP1 (SEQ ID No. 6) and Dm-AMP2 (SEQ ID No. 7).

Figure 15 shows a diagrammatic map of plasmid pAID-MR7.---

Please replace the paragraph on page 9, lines 22 through 31, with the following rewritten paragraph:

--- To amplify a 144 bp fragment of DNA encoding 48 amino acids of the mature Dm-AMP1 a PCR was carried out with Dahlia genomic DNA and oligonucleotides AFP-5 (based on Dm-AMP1 N-terminal amino acid sequence CEKASKTW) (SEQ ID NO: 13) and AFP-3EX (based on Dm-AMP1 C-terminal amino acid sequence MCFCYFNC) (SEQ ID NO: 14). Using the following conditions 94°C, 60 seconds, 48°C, 12 seconds and 72°C, 60 seconds for 35 cycles. A PCR product of approximately 150 bp was isolated from a 2% agarose gel by electroelution and ethanol precipitation. The PCR product was cloned into pBluescript by ligating blunt Bluescript vector and gel isolated PCR product together using T4DNA ligase and transforming into competent *E. coli* MC1022 cells. Transformation mixes were plated onto L-agar plates containing 100 µg/ml ampicillin and incubated at 37°C for 16 hours. Colonies were picked---

Please replace the paragraph on page 10, lines 5 through 10, with the following rewritten paragraph:

--- PCR clone 4 contained the DNA sequence
AAGACGTGGTCGGGAAACTGTGGCAATACGGG
ACATTGTGACAACCAATGTAAATCATGGGAGGGTGCGGCCCATGGAGCGT
GTCATGTGCGTAATGGGAAACACATGTGTTTCTGCTACTTCAAC (SEQ ID
NO: 15), encoding a portion of the observed mature Dm-AMP1 protein sequence
(KTWSGNCGNTGHCD
NQCKSWEGAAHGACHVRNGKHMCFYFN) (SEQ ID NO: 16).---

Please replace the section on page 10, lines 25 through 31, with the following rewritten section:

---Using the sequence of PCR clone 4 (above) and information from the NH₂ and COOH ends of the peptides described by cDNA sequences two pairs of oligonucleotides were designed for amplification of a gene encoding the observed mature Dm-AMP1.

A PCR was carried out with Dahlia genomic DNA and oligonucleotides MATAFP-5P (based on the codons present in Dm2.1, Dm2.3, Dm2.18 and Dm2.5 encoding the N-terminal amino acid sequence M(AV)(KN)(NR)SVAFF) (SEQ ID NO: 17) and MATAFP-5 (based on the mature Dm-AMP1 amino acid sequence NGKHMCF) (SEQ ID NO: 18) using the following conditions; 94°C, 60 seconds, 53°C,---

Please replace the paragraph on page 11, lines 5 through 12, with the following rewritten paragraph:

--- A PCR was carried out with Dahlia genomic DNA and oligonucleotides MATAFP-3 (based on the mature Dm-AMP1 amino acid sequence GACHVRN) (SEQ ID NO: 19) and DM25MAT-3 (based on the last two amino acids and the 3' untranslated region of Dm2.5) using the following conditions; 94°C, 60 seconds, 53°C, 12 seconds and 72°C, 60 seconds for 40 cycles. A PCR product of approximately 170 bp was isolated from a 2% agarose gel by electroelution and

ethanol precipitation. The PCR product was cloned into pBluescript and clones were sequenced as described above. A clone containing the 3' half of a Dm-AMP1 gene was identified.---

Please replace the section on page 13, lines 1 through 26, with the following rewritten section:

--- with Nco I. DNA sequencing confirmed that one transformant termed pDmAMPE, contained DNA encoding Dm-AMP leader, core and C-terminal extension. Both pDmAMPD and pDmAMPE vector sequences contained PCR derived base substitutions with respect to Dm-AMP1 gene DNA sequence however the base changes were silent having no effect on the expected amino acid sequence.

AFP-5 (to CEKASKTW) (SEQ ID NO: 13)

TG(T,C)GANAANGCN(A,T)(G,C)NAA(A,G)ACNTGG (SEQ ID NO: 20)

AFP-3EX (to MCFCYFNC) (SEQ ID NO: 14)

CA(A,G)TT(A,G)AANTANCANAAA(A,G)CACAT (SEQ ID NO: 21)

MATAFP-5P

ATGGC(C,G)AAN(A,C)(A,G)NTC(A,G)GTTGCNTT (SEQ ID NO: 22)

MATAFP-5

AAACACATGTGTTTCCCAT (SEQ ID NO: 23)

MATAFP-3

AGCGTGTCATGTGCGTAAT (SEQ ID NO: 24)

Dm25MAT-3

TAAAGAAACCGACCCTTTCACGG (SEQ ID NO: 25)

DMVEC-1

ATCGTAGCCATGGTGAATCGGTCGGTTGCGTTCTCCGCG (SEQ ID NO: 26)

DMVEC-2

AAACCGACCGAGCTCACGGATGTTCAACGTTTGGAAC (SEQ ID NO: 27)

DMVEC-3

ATGCATCCATGGTGAATCGGTCGGTTGCGTTCTCCGCGTTCGTTCTGATCC
TTTTCGTGCTCGCCATCTCAGATATCGCATCCGTTAGTGGAGAACTATGCG
AGAAA (SEQ ID NO: 28)

DMVEC-4

AGCAAGCTTTTCGGGAGCTCAACAATTGAAGTAA (SEQ ID NO: 29).---

Please replace the section on page 14, lines 22 through 31, with the following rewritten section:

--- Schematic representations of the plant transformation vectors used in this work, pFAJ3106 and pFAJ3109, are shown in figures 5 and 6, respectively. The nucleotide sequences comprised between the XhoI and SacI sites of these plasmids, which encompass the regions encoding antimicrobial proteins, are presented in Figures 7 and 8. The regions comprised between the XhoI and SacI sites of plasmid pFAJ3106 (shown in Figure 7) was constructed following the two-step recombinant PCR protocol of Pont-Kindom G.A.D. (1994, Biotechniques 16, 1010-1011). Primers OWB175 (5'AGGAAGTTCATTTTCATTTGG) (SEQ ID NO: 30) and OWB279 (5'-GCCTTTGGCACAACTTCTGCCTCTTTCCGATGAGTTGTTTCGGCTTTAAGTTT GTC) (SEQ ID NO: 31); were used in a first PCR reaction with plasmid pDMAMPE (see above) as a template. The second PCR reaction was done using as a template plasmid pFRG4 (Terras F.R.G. et al., 1995,---

Please replace the section on page 15, lines 1 through 11, with the following rewritten section:

--- Plant Cell 7, 573-588) and as primers a mixture of the PCR product of the first PCR reaction, primer OWB175 and primer OWB172 (5'TTAGAGCTCCTATTAACAAGGAAAGTAGC (SEQ ID NO: 32), SacI site underlined). The resulting PCR product was digested with XhoI and SacI and cloned into the expression cassette vector pMJB1 (see above). The expression cassette in the resulting plasmid, called pFAJ3099, was digested with HindIII (flanking the 5' end of the CaMV35S promoter) and EcoRI (flanking the 3' end of the nopaline synthase terminator) and cloned in the corresponding sites of the plant transformation vector pGPTVbar (Becker D. et al., 1992, Plant Mol. Biol. 20, 1195-1197) to yield plasmid pFAJ3106.

Plasmid pFAJ3109 was constructed by cloning the HindIII-EcoRI fragment of plasmid pDMAMPD (see above) into the corresponding sites of plant transformation vector pGPTVbar (see above).---